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# Predictors of Serum Chlorinated Pesticide Concentrations among Pre-Pubertal Russian Boys

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official opinion of the Centers for Disease Control and Prevention or of the National Institute for Environmental Health Sciences.

## ABSTRACT

**Background:** Few studies have evaluated predictors of childhood exposure to organochlorine pesticides (OCPs), a class of lipophilic persistent chemicals.

**Objectives:** To identify predictors of serum OCP concentrations -- hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and *p,p*-dichlorodiphenyldichloroethylene (*p,p*'-DDE) -- among boys in Chapaevsk, Russia.

**Methods:** Between 2003-2005, 499 boys ages 8-9 years were recruited in a prospective cohort. The initial study visit included a physical exam, blood collection, health, lifestyle, and food-frequency questionnaires, and determination of residential distance from a local factory complex that produced HCB and β-HCH. Fasting serum samples were analyzed for OCPs at the U.S. Centers for Disease Control and Prevention. General linear regression models were used to identify predictors of the boys' serum HCB, β-HCH, and *p,p'*-DDE concentrations.

**Results:** Among 355 boys with OCP measurements, median serum HCB, β-HCH, and *p,p'*-DDE concentrations were 158, 167, and 284 ng/g lipid respectively. Lower body mass index, longer breastfeeding duration, and local dairy consumption were associated with higher concentrations of OCPs. Boys who lived <2 km from the factory complex had 64% (95% CI: 37, 96) and 57% (95% CI: 32, 87) higher mean HCB and β-HCH concentrations than boys who lived ≥5 km away, respectively. Living >3 years in Chapaevsk predicted higher β-HCH concentrations, and having parents who lacked a high school education predicted higher *p,p'*-DDE concentrations.

**Conclusions:** Among this cohort of pre-pubertal Russian boys, predictors of serum OCPs included consumption of local dairy products, longer local residence, and residential proximity to the local factory complex.

# INTRODUCTION

Persistent, lipid-soluble organochlorine pesticides (OCPs) such as hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), dichlorodiphenyltrichlorethane (DDT) and its primary metabolite *p,p*'-dichlorodiphenyl-dichloroethylene (*p,p*'-DDE) are ubiquitous in the environment. Though these insecticides and fungicides were banned in the 1970s (U.S.) and in the 1980s (Russia) (Barber et al. 2005; Breivick et al. 1999; Jaga and Dharmani 2003), DDT is still used in some countries to control malaria and yellow fever (Jaga and Dharmani 2003), and HCB and β-HCH are generated as by-products during the manufacture of other chlorinated chemicals (Courtney 1979; Jung et al. 1997). In addition to biomagnifying up the food chain, these compounds have long half-lives in both the environment and the body, ranging from years to decades, and persist long after use has ceased (Barber et al. 2005; Courtney et al. 1979; Jaga and Dharmani 2003; Jung et al. 1997).

Human exposure typically occurs through diet (e.g., fatty fish, dairy products, meats, poultry) and less commonly through inhalation or dermal absorption (Darnerud et al. 2006; Gasull et al. 2011). OCPs are stored in adipose tissue, concentrated in breast milk (Rogan et al. 1986), and can be passed from mother to child via trans-placental transfer or breastfeeding, a primary route of exposure for children (Rogan et al. 1986; Sala et al. 2001). Compared to adults, young children have disproportionately elevated exposures to pesticides because they have higher ventilatory rates and food consumption (NRC 1993), and greater hand-to-mouth transfer of contaminated soil and dust. Moreover, the ability to metabolize, detoxify, and excrete pesticides may be reduced in young children (Landrigan et al. 2003).

HCB, β-HCH, and *p,p*'-DDE have been associated with adverse health effects in animal studies, including neurodevelopmental toxicity (Courtney 1979; Ecobichon et al. 1990), cancer (Courtney 1979; IARC 1987; IARC 1991), decreased male reproductive performance (e.g., mating index), and testicular abnormalities (Courtney 1979; Gralla et al. 1977; Gray et al. 2001; Van Velsen 1986). Among humans, there is evidence of neurodevelopmental toxicity following chronic exposure (reviewed in Ecobichon et al. 1990). Understanding the predictors of OCP exposure can improve child health and inform public health recommendations and policies to reduce childhood exposure. To date, some studies have evaluated predictors of childhood exposure to OCPs (Barr et al. 2006; Den Hond et al. 2009; Gallo et al. 2011; Karmaus et al. 2001; Lu et al. 2010; Petrik et al. 2006).

We assessed predictors of serum HCB, β-HCH, and *p,p*'-DDE concentrations among children residing in Chapaevsk, Russia, a city of approximately 72,000 people located 950 km southeast of Moscow with a history of environmental organochlorine compound contamination. In the 1930s, a complex of factories began producing chemical agents for military use (e.g., mustard gas, lewisite). From 1967 to 1987, production focused on organochlorine chemicals including HCB, HCH, and its derivatives (α-HCH, β-HCH, and γ-HCH) (Akhmedkanov et al. 2002). However, DDT was never produced (Sergeyev et al. 2008). By 2003, production of all chemicals ceased. Environmental contamination by HCB and β-HCH may have resulted from improper disposal, storage of hazardous waste from factories, or environmental release of organochlorine by-products of the manufacturing process (Shelepchikov et al. 2008).

## METHODS

**Study Population**. The Russian Children's Study is an ongoing prospective cohort study of 499 pre-pubertal boys in Chapaevsk, Russia. Briefly, 623 boys aged 8- and 9-years old were identified from the town-wide health insurance system. 572 were eligible and 516 agreed to participate (recruitment rate of 90%) though 17 boys were subsequently excluded due to their orphan status (precluding collection of residential history and other information). The first 144 9-year-old boys enrolled did not have OCPs measured, leaving 355 boys with serum OCP measurements. The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard School of Public Health, University of Massachusetts Medical School, and Brigham and Women's Hospital. The parent/guardian of each child gave informed consent and the children signed assent forms prior to participation.

At study entry, eligible boys had a physical examination and a fasting blood sample collected. A study nurse administered health and lifestyle questionnaires to the parent/guardian that included questions concerning the child's birth history, medical history, and physical activity; parental occupational and educational background; family medical history and residential history; and household income. The child's current residence was identified using an electronic map.

**Physical Examination.** At the initial visit, height and weight measurements were obtained according to a written protocol. Height in stocking feet was measured to the nearest 0.1 cm using a stadiometer. Weight was measured to the nearest 100 grams with a metric scale. Body mass index (BMI) was calculated as kilograms divided by squared height in meters.

**Blood Sample Analysis.** Fasting blood samples collected at study entry were centrifuged, and the serum was aliquoted and stored at -35°C until shipment on dry ice to the U.S. Centers for

Disease Control and Prevention (CDC) for analysis. Five OCPs (HCB, β-HCH, *p,p'*-DDE, aldrin, and mirex) were measured in the 355 boys. The samples, including method blank and quality control samples, were spiked with $^{13}C_{12}$-labeled pesticides, extracted by a C18 solid-phase extraction (SPE) followed by a multicolumn automated cleanup and enrichment procedure using either large-volume (Turner et al. 1997) or small-volume SPE (Sjodin et al. 2004). High-resolution mass spectrometry in selective ion monitoring was used to analyze the samples (Barr et al. 2003). Because aldrin and mirex were below the limit of detection (LOD) in almost all (over 95%) participants (median aldrin LOD of 0.40 ng/g lipid; median mirex LOD of 4.5 ng/g lipid), we limited our analysis to HCB, β-HCH, and *p,p'*-DDE, all of which were above the LOD in all samples (median HCB LOD of 4.1 ng/g lipid; median β-HCH LOD of 4.5 ng/g lipid; median *p,p*'-DDE LOD of 5.6 ng/g lipid). The analytical CVs for individual OCPs in QA/QC samples ranged between 10% and 15% over the course of the study. All OCP concentrations were expressed on a per-lipid basis. Total serum lipid content was determined from enzymatic measurements of total cholesterol and triglycerides (Phillips et al. 1989).

**Distance Information.** The straight-line distance from each participant's residence to the primary factory complex was calculated using ArcView GIS 3.0 (ESRI, Redlands, CA, USA) by the Moscow Ecological Analytical Center.

**FFQ.** Study nurses administered a validated Russian Institute of Nutrition (RIN) food frequency questionnaire (FFQ) that was modified to assess local food consumption (Hauser et al. 2005). The FFQ included questions on the usual frequency of consumption and portion sizes (classified based on photographs) of >70 food items consumed during the previous year (Martinchik et al. 1998). The participating boy and his parent/guardian completed the FFQ together.

**Statistical Analysis.** Spearman correlations were used to assess relationships among OCPs. Linear regression models were used to identify predictors of the serum concentration of each OCP. To improve normality and reduce the influence of outliers, serum OCP concentrations were $\log_{10}$-transformed. Potential predictors were identified using *a priori* knowledge from the literature. All covariates (except food consumption) with $p \leq 0.20$ in bivariate analyses were included in an initial multivariable linear regression model for each OCP that was subsequently reduced to include predictors with $p \leq 0.10$ only. In addition, breastfeeding duration was retained in all models because it is a known route of exposure for children and there was a significant linear trend ($p \leq 0.05$) in the concentration of all three OCPs over categories of breastfeeding duration. Breastfeeding duration was categorized as none, 1-13 weeks, and >13 weeks to limit the potential influence of a small number of observations with a long duration of breastfeeding. To account for non-linearity, BMI was grouped according to WHO child growth standards: underweight (<1 SD below the mean), normal, and overweight/obese (>1 SD above the mean) (de Onis et al. 2007). We modeled other continuous predictors as simple continuous variables and as categorical variables, and examined point estimates and compared the goodness-of-fit of each model based on adjusted R-square values to determine the most appropriate coding.

Individual food items were categorized in to groups (eggs, dairy products, poultry, non-poultry meats, fish, and fruits and vegetables) and the usual frequency and portion size (grams/year) of each category was determined. In addition, we estimated consumption of local foods in each food category, and included each local food category [in tertiles (low, medium, or high) or dichotomized as any versus not when <50% reported eating local foods] in multivariable models while simultaneously adjusting for total consumption of each food category (in tertiles). Local foods reflect possible local contamination while total foods are adjusted in models to reflect

background levels. Because of the specific interest in dietary sources of OCP concentrations, each food group was considered separately as well as simultaneously, adjusting for other factors.

For ease of interpretation, regression coefficients are presented as the estimated percent change in the serum concentration of each OCP with a one-unit increase in exposure (for continuous variables) or relative to the reference category (for categorical variables) ($10^{\beta}$, where $\beta$ is the regression coefficient for a given predictor) holding all other variables constant. We also estimated adjusted mean concentrations of each OCP according to residential distance from the factory using least square means, adjusted for all other predictors in the final model for each OCP. Additionally, we performed sensitivity analyses using $\log_{10}$-transformed whole-weight serum OCP concentrations adjusted for total lipids instead of direct-lipid adjusted OCPs in the final model. A p-value of $<0.05$ was considered statistically significant. Tests for trend were performed by modeling categorical variables as an ordinal variable using integer values (0, 1, 2). All data analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

**Study Population.** Characteristics of the 355 boys are shown in Table 1. At study entry, 84% of boys were 8 years old, 17% were overweight/obese, and 25% were underweight (de Onis et al. 2007). Among boys with and without serum OCP measurements (n=355 vs. 144), there were no significant differences in height, weight, BMI z-scores, birth or family characteristics, but there was a significant difference in household income (44% vs. 26% of families in the highest income category, respectively). Also more boys with measured serum OCP concentrations had fathers who were employed at the factory complex compared to those without OCP measurements (14% vs. 9%).

**Distribution of serum HCB, β-HCH, *p,p'*-DDE.** The medians ($25^{th}$, $75^{th}$ percentiles) for serum HCB, β-HCH, and *p,p*'-DDE concentrations were 158 (107, 246), 167 (112, 270), and 284 (187, 492) ng/g lipid, respectively (Table 2). Median *p,p*'-DDE concentrations were about three times higher than concentrations previously reported for adolescents in the U.S. and Belgium, while HCB concentrations were about 12 times higher (Table 3) (Den Hond et al. 2011; Patterson et al. 2009). The median β-HCH concentration previously reported for 12-19 year old U.S. adolescents (below the LOD of 7.8 ng/g lipid) (Patterson et al. 2009) was at least 20 times lower than the median concentration in our study population of Russian boys. Spearman correlations between the OCPs were r = 0.61 for β-HCH and *p,p*'-DDE, r = 0.54 for β-HCH and HCB, and r = 0.34 for HCB and *p,p*'-DDE.

**Predictors of serum HCB, β-HCH, *p,p'*-DDE.** BMI and residential distance from the factory complex were the strongest predictors, explaining 18% and 23% of the variability in the serum concentrations of HCB and β-HCH, respectively. All other covariates combined explained an additional 3% (HCB) and 13% (β-HCH) of the variability. For *p,p*'-DDE, BMI and breastfeeding duration combined explained 23% of the variability, all other model covariates explained an additional 7%.

Boys breastfed >13 weeks had 16% (95% CI: -5, 41%), 63% (95% CI: 35, 96%), and 81% (95% CI: 43, 128%) higher predicted mean serum HCB, β-HCH, *p,p*'-DDE concentrations, respectively, than non-breastfed boys, with a significant linear trend over increasing categories of breastfeeding for all three OCPs (Table 4). BMI was also a significant predictor of all three OCPs, with the highest mean concentrations among boys who were classified as underweight, and the lowest among boys who were overweight or obese. Living near the factory complex predicted increased serum concentrations of HCB (9.3%; 95% CI: -4.9, 25.6% and 64%; 95%

CI: 37, 96% for 2 to <5 km and <2 km compared with ≥5 km, respectively), and β-HCH (33.7%; 95% CI: 17.1, 52.7% and 57.1%; 95% CI: 32, 87%, respectively.) Living 2 to <5 km from the factory was a significant predictor of serum *p,p*'-DDE concentrations (35.6%; 95% CI: 15.1, 59.8%), while predicted concentrations were increased but lower for boys living <2 km from the factory (16.7%; 95% CI: -5.2, 43.7%)(Table 4). Specifically, for boys living < 2km from the factory complex, these estimated increases correspond to mean HCB, β-HCH, and *p,p*'-DDE serum concentrations of 223 ng/g lipid (95% CI: 191, 260 ng/g lipid), 208 ng/g lipid (95% CI: 181, 240 ng/g lipid), and 284 ng/g lipid (95% CI: 233, 346 ng/g lipid), respectively, adjusted for the final model covariates for each OCP (Figure 1).

Any local dairy consumption (vs. none) predicted higher HCB, β-HCH, and *p,p*'-DDE serum concentrations of 14% (95% CI: 0.6, 30%), 21% (95% CI: 7, 36%), and 18% (95% CI: 1, 37%) respectively, even with adjustment for total dairy consumption (which was not a significant predictor of any of the OCPs) (Table 4). Although any local poultry consumption predicted higher HCB ($p = 0.09$) and total egg consumption predicted higher β-HCH ($p=0.09$), only local dairy consumption was a significant predictor when poultry, egg, and dairy consumption were modeled simultaneously (data not shown).

Factors that predicted higher concentrations of only one of the OCPs were having parents who lacked a high school education (57% higher serum *p,p*'-DDE relative to boys whose parents had university/post-graduate training, 95% CI: 17, 111%), living in Chapaevsk for ≥3 years (higher β-HCH concentrations compared to those living <3 years in Chapaevsk, trend $p = 0.003$) and having a father employed at the factory complex (higher β-HCH concentrations, $p = 0.10$).

The percent of variation ($R^2$) explained by the predictors in each final OCP model ranged from 0.21 (for the model of HCB) to 0.36 (for β-HCH). Sensitivity analyses of predictors for whole weight of $log_{10}$-transformed serum OCPs adjusted for total lipids were consistent with predictions of lipid-adjusted serum OCPs (Supplemental Material, Table S1).

## DISCUSSION

In the present study, we measured serum OCP concentrations and identified several demographic, lifestyle, and environmental predictors among boys living in Chapaevsk, Russia, a town contaminated by previous industrial activity. These results complement a publication describing predictors of dioxins and polychlorinated biphenyls (PCBs) among these boys (Burns et al. 2009). Despite the young age of our cohort (8-9 years), concentrations of OCPs were similar to or higher than concentrations reported for somewhat older pediatric populations (range: 8-19 years) in the U.S. and Europe (Barr et al. 2006; Patterson et al. 2009; Den Hond et al. 2011; Petrik et al. 2006).

Consistent with other studies of persistent organic pollutants in this cohort and other populations (Burns et al. 2009; Den Hond et al. 2009; Gallo et al. 2011; Humblet et al. 2010; Karmaus et al. 2001), lower BMI predicted higher serum OCP concentrations. This finding may be due to a smaller volume of distribution in boys with lower BMI resulting in higher serum concentrations (Wolff et al. 2005).

Breastfeeding is a known route of early life exposure to lipophilic persistent compounds (Rogan et al. 1986). Consistent with other studies (Barr et al. 2006; Den Hond et al. 2009; Gallo et al. 2011; Karmaus et al. 2001), longer breastfeeding duration (>13 weeks) predicted higher OCP

concentrations. Although breastfeeding in our cohort ended years before OCP measurement, childhood concentrations of lipophilic compounds track closely with breastfeeding exposure in infancy (Patandin et al. 1999).

Residential distance from the primary factory may provide insight on chemical-specific pathways of exposure in this area. Specifically, living <2 km from the complex was associated with higher serum HCB and β-HCH concentrations, which is consistent with these compounds having been manufactured at the factory as a source of exposure. However, serum *p,p*'-DDE levels were highest for boys living 2 to <5 km from the factory and only moderately and non-significantly elevated among boys living within 2 km. DDT, the parent compound for *p,p*'-DDE, was not manufactured at the complex, and other exposure sources likely contributed to the boys' *p,p*'-DDE levels.

Duration of residence in Chapaevsk and father's prior employment at the factory were positive predictors of β-HCH. We hypothesized that longer residence in Chapaevsk would be associated with higher exposure to and bioaccumulation of both β-HCH and HCB, since both were produced locally. Therefore, it is unclear why duration of residence was a significant predictor of β-HCH but not HCB. Similarly, fathers' prior occupation at the factory was not a significant predictor of HCB, despite the same potential for exposure from residues on the fathers' work clothing, boots, tools, or skin (Lu et al. 2000). Mother's employment at the factory did not predict any of the OCPs, but only 5% of mothers reported previous employment at the factory, limiting power to detect a statistically significant association.

Non-occupational exposure to HCB, β-HCH, and *p,p*'-DDE is primarily dietary (Darnerud et al. 2006; Gasull et al. 2011, therefore, we expected consumption of local foods high in fat, such as

dairy and fish, to predict higher serum concentrations. However, local dairy consumption was the only significant predictor of all three OCPs. Studies of OCP exposure and local diet among children have been limited to two assessments concerned about local environmental contamination (Den Hond et al. 2009; Gallo et al. 2011), that did not find an association with local dairy. Dairy consumption has been associated with serum OCP concentrations in several adult populations, although none differentiated whether dairy were from local sources (Arrebola et al. 2009; Arrebola et al. 2012; Lee et al. 2007). Although previous studies among children reported associations between serum OCPs and consumption of local fish (Gallo et al. 2011), and consumption of fatty meats and vegetables (Den Hond et al. 2009), these foods were not significant predictors in our cohort.

We previously reported that consumption of most local foods predicted higher serum dioxins and PCBs in the same study cohort (Burns et al. 2009), consistent with findings reported for other study populations concerned about environmental contamination (Choi et al. 2006; Gallo et al. 2011; Schecter et al. 2003). It is unclear why local food consumption, apart from dairy, was associated with dioxin-like compounds, but not serum OCP concentrations, in our study cohort.

One limitation of our diet analyses is the inability to assess consumption of specific types of fish or fat content. We had only father's reported employment history with no independent verification, and therefore can only speculate on the association observed with β-HCH. While we attempted to evaluate many potential determinants of these exposures (e.g., parental education), there were probably other predictors that we could not assess.

A major strength of this study is the large sample size of young boys with serum HCB, β-HCH, and *p,p*'-DDE measurements. For all three OCPs, all serum concentrations were above the limit

of detection with wide ranges of concentrations. Detailed dietary information, including local food consumption, as well as calculated residential distance from the factory complex, was also available. In this context, this study contributes to understanding of determinants of serum OCP levels among children, and in particular, highlights the potential importance of local risk factors for exposure.

## CONCLUSION

Our findings suggest that contamination from the local factory may be an important source of HCB and β-HCH exposure for boys in Chapaevsk. Residential distance from the primary factory was a significant predictor of serum HCB and β-HCH, both of which were manufactured at the complex. Father's past employment at the factory and longer residence in Chapaevsk also predicted higher serum β-HCH, and local dairy consumption predicted higher serum concentrations of all three OCPs, which adds further support to local environmental contamination, at least partly from the factory, as a source of exposure. Consistent with other studies, longer breastfeeding duration and lower BMI predicted higher serum OCPs.

Our findings provide insight on determinants of OCP exposure, which may lead to local monitoring and continuation of remediation measures (e.g., soil removal) to reduce childhood and community exposure. While our findings suggest that local dairy consumption and longer breastfeeding duration are primary determinants of OCP exposure, it would be premature to recommend reduced intake of local dairy or breastfeeding without fully understanding the exposure pathway and the risk-benefit trade-offs from such a recommendation. It is important to keep in mind that these local food products were central to the children's diet in this region, and that breastfeeding has well-established benefits. Recommendations to prevent childhood

exposure include environmental clean-up of contaminated areas, regulatory enforcement of safe practices for industrial waste disposal and emissions control, and preferential consumption, when available, of foods produced in non-contaminated areas.

## References


Akhmedkanov A, Revich B, Adibi JJ, Zeilert V, Masten SA, Patterson DG Jr, et al. 2002. Characterization of dioxin exposure in residents of Chapaevsk, Russia. J Exposure Analysis and Environmental Epidemiology 12:409-417.

Arrebola JP, Martin-Olmedo P, Fernandez MF, Sanchez-Cantalejo E, Jimenez-Rios JA, Torne P, et al. 2009. Predictors of concentrations of hexachlorobenzene in human adipose tissue: a multivariate analysis by gender in Southern Spain. Environ Int 35:27-32.

Arrebola JP, Mutch E, Rivero M, Choque A, Silvestre S, Olea N, et al. 2012. Contribution of sociodemographic characteristics, occupation, diet and lifestyle to DDT and DDE concentrations in serum and adipose tissue from a Bolivian cohort. Environ Int 38:54-61.

Barber JL, Sweetman AJ, van Wijk D, Jones KC. 2005. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends, and processes. Sci Total Environ 349:1-44.

Barker DJ. 1995. Fetal origins of coronary heart disease. BMJ 311:171-174.

Barr DB, Weihe P, Davis MD, Needham LL, Grandjean P. 2006. Serum polychlorinated biphenyl and organochlorine insecticide concentrations in a Faroese birth cohort. Chemosphere 62:1167-1182.

Barr JR, Maggio VL, Barr DB, Turner WE, Sjodin A, Sandau CD, et al. 2003. New high-resolution mass spectrometric approach for the measurement of polychlorinated biphenyls and organochlorine pesticides in human serum. J Chromatogr B Analyt Technol Biomed Life Sci 794:137-148.

Breivik K, Pacyna JM, Munch J. 1999. Use of α-, β-, and γ-hexachlorocyclohexane in Europe, 1970-1996. Sci Total Environ 239:151-163.

Burns JS, Williams PL, Sergeyev O, Korrick S, Lee MM, Revich B, et al. 2009. Predictors of serum dioxins and PCBs among peripubertal Russian boys. Environ Health Perspect 117:1593-1599.

Choi AL, Levy JI, Dockery DW, Ryan LM, Tolbert PE, Altshul LM et al. 2006. Does living near a Superfund site contribute to higher polychlorinated biphenyl (PCB) exposure? Environ Health Perspect 114:1092-1098.

Courtney KD. 1979. Hexachlorobenzene (HCB): a review. Environ Res 20:225-266.

Darnerud PO, Atuma S, Aune M, Bjerselius R, Glynn A, Grawe KP, et al. 2006. Dietary intake estimations of organohalogens contaminants (dioxins, PCB, PBDE, and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. Food Chem Toxicol 44:1597-1606.

de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 85:660-667.

Den Hond E, Govarts E, Bruckers L, Schoeters G. 2009. Determinants of polychlorinated aromatic hydrocarbons in serum in three age class—methodological implications for human biomonitoring. Environ Res 109:495-502.

Den Hond E, Dhooge W, Bruckers L, Schoeters G, Nelen V, van de Mieroop E, et al. 2011. Internal exposure to pollutants and sexual maturation in Flemish adolescents. J Expo Sci Environ Epidemiol 21:224-233.

Ecobichon, DJ, Davies JE, Doull J, Ehric M, Joy R, McMillan R, et al. 1990. Neurotoxic effects of pesticides. In The Effect of Pesticices on Human Health. Princeton Scientific Pub. Co., Inc.: Princeton, NJ. CF Wilkinson and SR Baker eds. 131-199.

Gallo MV, Schell LM, DeCaprio AP, Jacobs A. 2011. Levels of persistent organic pollutant and their predictors among young adults. Chemosphere 83:1374-1382.

Gasull M, Bosch de BM, Puigdomenech E, Pumarega J, Porta M. 2011. Empirical analyses of the influence of diet on human concentrations of persistent organic pollutants: a systematic review of all studies conducted in Spain. Environ Int 37:1226-1235.

Gralla EJ, Fleischman RW, Luthra YK, Haggpian M, Baker JR, et al. 1977. Toxic effects of hexachlorobenzene after daily administration to beagle dogs for one year. Toxicol Appl Pharmacol 40:227-239.

Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L, et al. 2001. Effects of environmental antiandrogens on reproductive development in experimental animals. Human Reprod Update 7:248-264.

Hauser R, Williams P, Altshul L, Korrick S, Peeples L, Patterson DG Jr, et al. 2005. Predictors of serum dioxin levels among adolescent boys in Chapaevsk, Russia: A cross-sectional pilot study. Environ Health 4:8.

Humblet O, Williams PL, Korrick SA, Sergeyev O, Emond C, Birnbaum LS, et al. 2010. Predictors of serum dioxin, furan, and PCB concentrations among women from Chapaevsk, Russia. Environ Sci Technol 44:5633-5640.

IARC (International Agency for Research on Cancer). 1987. Hexachlorocyclohexanes (Group 2B). In Some halogenated hydrocarbons. IARC monographs on the evaluation of carcinogenic risks to humans. Supplement 7. Lyon, IARC. 220-222.

IARC (International Agency for Research on Cancer). 1991. DDT and associated compounds. In Occupational exposures in insecticide application, and some pesticides. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, IARC. 179-249.

Jaga K and Dharmani C. 2003. Global surveillance of DDT and DDE levels in human tissues. Int J Occup Med Environ Health 16:7-20.

Jung D, Becher H, Edler L, Flesch-Janys D, Gurn P, Konietzko J, et al. 1997. Elimination of β-Hexachlorocyclohexane in occupationally exposed persons. J Toxicol Environ Health 51:23-34.

Karmaus W, DeKoning EP, Kruse H, Witten J, Osius N. 2001. Early childhood determinants of organochlorine concentrations in school-aged children. Pediatr 50:331-336.

Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. 2003. Children's health and the environment: public health issues and challenges for risk assessment. Environ Health Perspect 112:257-265.

Lee SA, Dai Q, Zheng W, Gao YT, Blair A, Tessari JD, et al. 2007. Association of serum concentration of organochlorine pesticides with dietary intake and other lifestyle factors among urban Chinese women. Environ Int 33:157-163.

Lu Y, Zhou SB, Li BX. 2010. Exposure to environmental hexachlorohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) among rural children in north eastern China. Biomed Environ Sci 23:230-233.

Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res 84:290-302.

Martinchik AN, Baturin AK, Baeva VS, Feoktistova AI, Piatnikskaia IN, Azizbekian GA, et al. 1998. Development of a method of studying actual nutrition according to analysis of the frequency of consumption of food products: creation of a questionnaire and general evaluation of the reliability of the method [in Russian]. Vopr Pitan 3:8-13.

NRC (National Research Council). 1993. "FRONT MATTER." *Pesticides in the Diets of Infants and Children*. Washington, DC: The National Academies Press.

Patandin S, Dagnelie PC, Mulder PG, Op de Coul E, van der Veen JE, Weisglas-Kuperus N, et al. 1999. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: a comparison between breast-feeding, toddler, and long-term exposure. Environ Health Perspect 107:45-51.

Patterson DG Jr., Wong LY, Turner WE, Caudill SP, Dipietro ES, McClure PC, et al. 2009. Levels in the U.S. population of those persistent organic pollutants (2003-2004) included in the Stockholm Convention or in other long range transboundary air pollution agreements. Environ Sci Technol 43:1211-1218.

Petrik J, Drobna B, Pavuk M, Jursa S, Wimmerova S, Chovancova J. 2006. Serum PCBs and organochlorine pesticides in Slovakia: age, gender, and residence as determinants of organochlorine concentrations. Chemosphere 65:410-418.

Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 18:495-500.

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, et al. 1986. Polychlorinated biphenyls (PCBs) and Dichlorodiphenyl Dichloroethene (DDE) in human milk: effects of maternal factors and previous lactation. Amer J Public Health 76:172-177.

Sala M, Ribas-Fito N, Cardo E, de Muga ME, Marco E, Mazon C, et al. 2001. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. Chemosphere 43:895-901.

Schecter A, Quynh HT, Pavuk M, Papke O, Malisch R, Constable JD. 2003. Food as a source of dioxin exposure in the residents of Bien Hoa City, Vietnam. J Occup Environ Med 45(8):781-788.

Sergeyev O, Shelepchikov A, Denisova T, Revich B, Saharov I, Sotskov Y, et al. 2008. POPs in human milk in Chapaevsk, Russia, five years following cessation of chemical manufacturing and decade of remediation program, pilot study. Oranohalogen Compounds 70:1946-1949.

Shelepchikov A, Sergeyev O, Revich B, Saharov I, Sotskov Y, Brodsky E, et al. 2008. Chlorine industry in the former USSR, Chapaevsk, Russia. Organohalogen Compounds 70:1950-1953.

Sjodin A, Jones RS, Lapeza CR, Focant JF, McGahee EE 3rd, Patterson DJ Jr. 2004. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Anal Chem 76:1921-1927.

Turner W, DiPietro E, Lapeza C, Green V, Gill J, Patterson DGJ. 1997. A fast universal automated cleanup system for the isotope-dilution high-resolution mass spectrometric analysis of PCDDs, PCDFs, coplanar PCBs, PCB congeners, and persistent pesticides from the same serum sample. Organohalogen Compounds 31:26-31.

Van Velsen FL, Danse LH, Van Leeuwen FX, Dormans JA, Van Logten MJ. 1986. The subchronic oral toxicity of the beta-isomer of hexachlorocyclohexane in rats. Fundam Appl Toxicol 6:697-712.

Wolff MS, Britton JA, Teitelbaum SL, Eng S, Deych E, Ireland K, et al. 2005. Improving organochlorine biomarker models for cancer research. Cancer Epidemiol Biomarkers Prev 14:2224-36.

Table 1. Characteristics of 8-9 year old participants in the Russian Children's Study at study entry

| Characteristic | Total boys (n=355) |
|---|---|
| **Growth Measurements** | |
| Height (cm) | 129 ± 6.04 |
| Weight (kg) | 26.7 ± 5.53 |
| Body Mass Index (BMI) | 15.9 ± 2.33 |
| WHO Height z-score | 0.13 ± 1.01 |
| WHO BMI z-score | -0.16 ± 1.31 |
| **Birth and Neonatal History** | |
| Birth Weight (kg) | 3.3 ± 0.53 |
| Gestational Age (wks) | 39.0 ± 1.81 |
| Duration of Breastfeeding (wks) | |
| None | 46 (13) |
| 1-13 wks | 143 (40) |
| >13 wks | 160 (45) |
| **Boys Dietary Consumption of Any Local Foods** | |
| Dairy | 151 (42) |
| Poultry | 29 (8) |
| Non-Poultry Meats | 20 (6) |
| Fish | 76 (21) |
| Eggs | 54 (15) |
| Fruits and Vegetables | 341 (96) |
| **Parental and Residential Characteristics** | |
| Duration of Chapaevsk Residence | |
| <3 yrs | 101 (28) |
| 3 to <6 yrs | 71 (20) |
| 6 to <8 yrs | 94 (26) |
| ≥ 8 yrs | 87 (25) |
| Any Household Smoking During Pregnancy | 59 (17) |
| Mother ≤ 25 years old at Son's Birth | 248 (70) |
| Maximum Parental Education | |
| High School or Less | 30 (8) |
| Jr College/Technical School | 201 (57) |
| University/Post-Graduate Training | 122 (34) |
| Household Income, USD per month | |
| <175 | 110 (31) |
| 175-250 | 89 (25) |
| >250 | 155 (44) |
| Father Employed at Factory Complex | 50 (14) |
| Residential Distance to Factory Complex | |
| <2 km | 65 (18) |
| 2 to <5 km | 159 (45) |
| ≥5 km | 131 (37) |

Data are mean ± SD, or n (%) unless stated otherwise

**Missing**: Birth weight, n=1; Gestational age, n=2; Breastfeeding duration, n=6; Local dairy consumption, n=3; Local poultry consumption, n=3; Local non-poultry meat consumption, n=3; Local fish consumption, n=3;

Local egg consumption, n=5; Local fruit and vegetables, n=3; Duration of Chapaevsk residence, n=2; Mother's age at son's birth, n=3; Maximum parental education, n=2; Household income, n=1; Any household smoking during pregnancy, n=5; Father employed at factory, n=20. All percentages are based on available data excluding missing observations.

Table 2. Distribution of measured OCPs (ng/g lipid) among 8- and 9- year old boys enrolled in the Russian Children's Study (n=355)[a]

| **OCP** | N | **Percentile** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Min | 10th | 25th | 50th (median) | 75th | 90th | Max |
| HCB | 355 | 32 | 80 | 107 | 158 | 246 | 364 | 2660 |
| β-HCH | 355 | 39 | 81 | 112 | 167 | 270 | 412 | 2860 |
| *p,p*'-DDE | 355 | 49 | 122 | 187 | 284 | 492 | 835 | 9370 |

[a] No values below the limit of detection (LOD)

Table 3. Median organochlorine pesticide concentrations (ng/g lipid) in 8- to 9-year-old boys from Chapaevsk, Russia compared to other pediatric studies

| **Country** | **Year** | **n** | **Age Range (yrs)** | **Population** | **HCB** | **β-HCH** | ***p,p*'-DDE** |
|---|---|---|---|---|---|---|---|
| Russia (current study) | 2005-2006 | 355 | 8-9 | Boys | 158 | 167 | 284 |
| USA (NHANES)[a] | 2003-2004 | 588 | 12-19 | Boys and Girls | 13.4 | <LOD | 93.6 |
| Belgium[b] | 2003-2004 | 1679 | 14-15 | Boys | 22.8 | -- | 104 |
| Faroe Islands[c] | 1986-1987 | 788 | 14 | Boys and Girls | -- | -- | 467 |
| Slovakia[d] (contaminated Michalovce district) | 2001 | 216 | 8-10 | Boys and Girls | 79.6 | -- | 344 |

-- OCP not measured

Limit of detection (LOD) = 7.8 ng/g lipid

HCB: Hexachlorobenzene; β-HCH: β-hexachlorocyclohexane; *p,p*'-DDE: *p,p*-dichlorodiphenyldichloroethylene

[a] Patterson et al. 2009

[b] Den Hond et al. 2011

[c] Barr et al. 2006

[d] Petrik et al. 2006

Table 4. Final multivariable predictor models for serum concentrations of organochlorine pesticides based on linear regression models

| | HCB (n=346) | | β-HCH (n=327) | | *p,p*'-DDE (n=346) | |
|---|---|---|---|---|---|---|
| | Estimated % Change in Pesticide (95% CI)* | p-value[#] | Estimated % Change in Pesticide (95% CI)* | p-value[#] | Estimated % Change in Pesticide (95% CI)* | p-value[#] |
| **WHO BMI Z categories** | | | | | | |
| Underweight | 28.3 (10.6, 48.8) | 0.001 | 18.6 (3.1, 36.5) | 0.02 | 9.0 (-8.5, 29.8) | 0.34 |
| Normal | REF | | REF | | REF | |
| Overweight/Obese | -36.1 (-46.3, -23.9) | <0.001 | -44.1 (-52.5, -34.2) | <0.001 | -51.0 (-60.0, -39.9) | <0.001 |
| *p for trend* | | <0.001 | | <0.001 | | <0.001 |
| **Breastfeeding duration** | | | | | | |
| None | REF | | REF | | REF | |
| 1-13 wks | 2.0 (-16.3, 24.4) | 0.84 | 13.2 (-6.4, 36.8) | 0.20 | 8.0 (-14.4, 36.4) | 0.52 |
| >13 wks | 15.9 (-4.8, 41.0) | 0.14 | 62.8 (35.0, 96.3) | <0.001 | 81.0 (43.3, 128.4) | <0.001 |
| *p for trend* | | 0.05 | | <0.001 | | <0.001 |
| **Residential distance from factory complex** | | | | | | |
| <2 km | 63.8 (37.0, 95.9) | <0.001 | 57.1 (31.8, 87.2) | <0.001 | 16.7 (-5.2, 43.7) | 0.15 |
| 2 to <5 km | 9.3 (-4.9, 25.6) | 0.21 | 33.7 (17.1, 52.7) | <0.001 | 35.6 (15.1, 59.8) | <0.001 |
| ≥5 km | REF | | REF | | REF | |
| *p for trend* | | <0.001 | | <0.001 | | 0.03 |
| **Local dairy consumption** | 14.4 (0.6, 30.1) | 0.04 | 20.6 (6.9, 36.1) | 0.003 | 17.5 (1.0, 36.7) | 0.04 |
| **Total dairy consumption**[a] | | | | | | |
| Low | REF | | REF | | REF | |
| Medium | -2.7 (-16.6, 13.5) | 0.72 | -2.9 (-16.1, 12.3) | 0.69 | -7.5 (-22.7, 10.8) | 0.40 |
| High | -0.7 (-15.0, 16.1) | 0.93 | -2.2 (-15.5, 13.2) | 0.76 | -10.7 (-25.8, 7.4) | 0.23 |
| *p for trend* | | 0.93 | | 0.76 | | 0.23 |
| **Duration of Chapaevsk Residence** | | | | | | |
| <3 yrs | --- | --- | REF | | --- | --- |
| 3 to <6 yrs | --- | --- | 28.3 (7.9, 52.5) | 0.005 | --- | --- |
| 6 to <8 yrs | --- | --- | 28.0 (9.1, 50.2) | 0.003 | --- | --- |
| ≥8 yrs | --- | --- | 28.0 (9.0, 50.4) | 0.003 | --- | --- |
| *p for trend* | --- | --- | | 0.003 | --- | --- |

| | HCB (n=346) | | β-HCH (n=327) | | *p,p*'-DDE (n=346) | |
|---|---|---|---|---|---|---|
| | Estimated % Change in Pesticide (95% CI)* | p-value[#] | Estimated % Change in Pesticide (95% CI)* | p-value[#] | Estimated % Change in Pesticide (95% CI)* | p-value[#] |
| **Father worked at factory complex** | --- | --- | 16.0 (-2.6, 38.0) | 0.10 | --- | --- |
| **Maximum parental education** | --- | --- | --- | --- | | |
| High school or less | --- | --- | --- | --- | 57.2 (16.9, 111.4) | 0.003 |
| Junior college/Technical school | --- | --- | --- | --- | 8.1 (-8.0, 27.1) | 0.34 |
| University/Post-Graduate | --- | --- | --- | --- | REF | |
| *p for trend* | --- | --- | --- | --- | | 0.01 |
| Total model R-square | 0.21 | | 0.36 | | 0.30 | |

*Estimated change in pesticide concentration based on β parameter estimates for predicting log (base 10) lipid-adjusted concentrations and then calculating $10^{\beta}$

# p-value is from Wald statistic

[a]Total dairy consumption is included in final models to reflect background levels

Final models include predictors with p≤0.10

## Figure Legend

**Figure 1.** Adjusted mean OCP serum concentrations of Russian pre-pubertal boys in relation to residential distance from factory complex. Adjusted means (95% CI) calculated using least square means adjusted for all other covariates in Table 3. HCB: Hexachlorobenzene; β-HCH: β-Hexachlorocyclohexane; *p,p*'-DDE: *p,p*-dichlorodiphenyldichloroethylene.

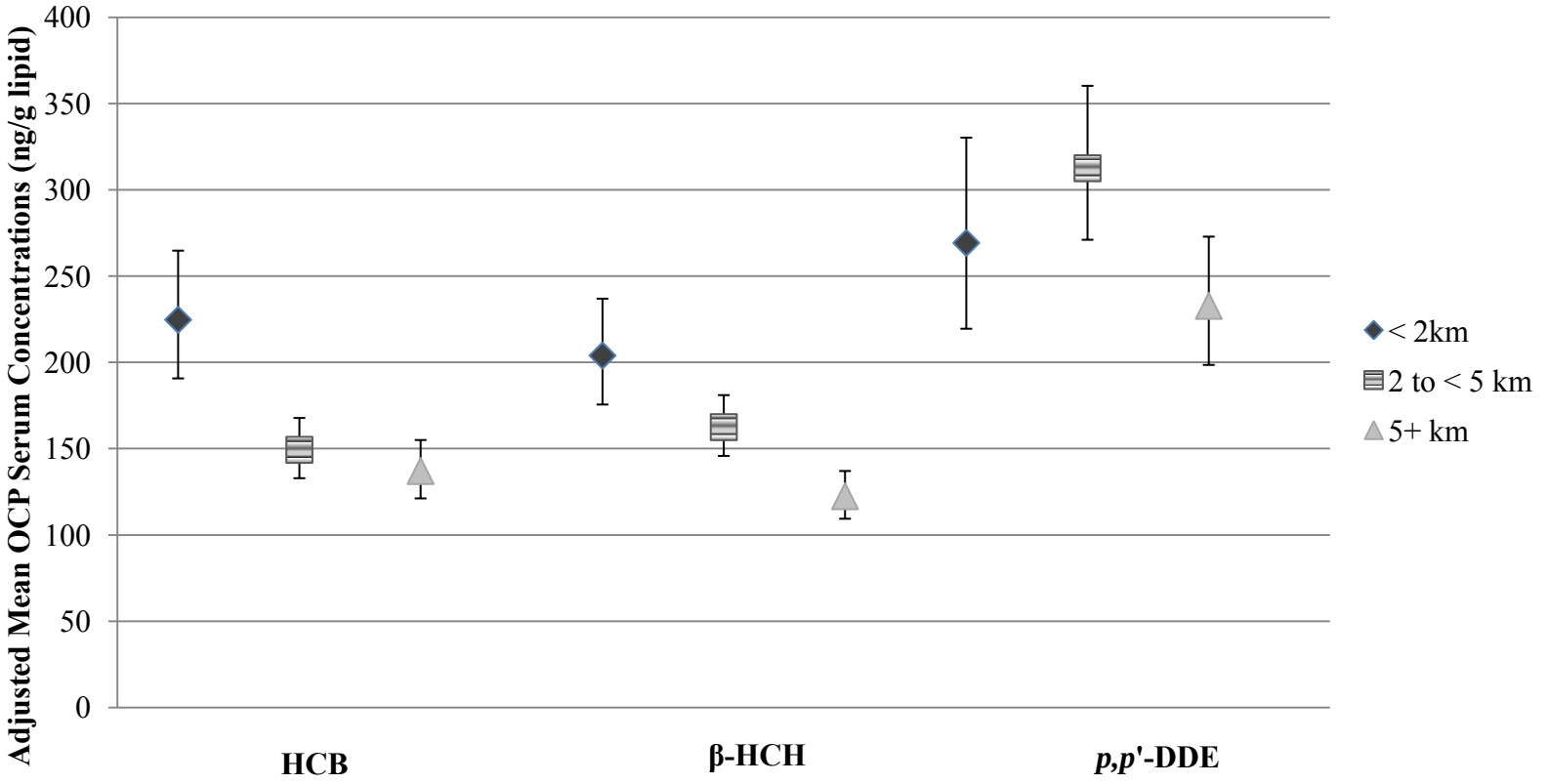
Adjusted Mean OCP Serum Concentrations (ng/g lipid)
400
350
300
250
200
150
100
50
0
HCB
β-HCH
p,p'-DDE
< 2km
2 to < 5 km
5+ km

Figure 1.